ABSTRACT

For several decades, antiangiogenic drug therapies have been pursued in oncology as a way of controlling angiogenesis in tumors to prevent their growth and metastasis. In ophthalmology, such investigations have been more recent, with the focus on treating ocular angiogenesis as a way of preventing vision loss. The angiogenic factor attracting the most attention for the treatment of choroidal neovascularization in age-related macular degeneration (AMD) is vascular endothelial growth factor (VEGF). This article reviews the properties of VEGF, its role in neovascular AMD, and the clinical challenges of blocking angiogenesis. This summary also reports on antiangiogenic agents, focusing primarily on investigational agents in the treatment of AMD. The recently approved antiangiogenic drug, pegaptanib, is described briefly but treated in more detail elsewhere in this monograph. (Adv Stud Ophthalmol. 2005;2(2):62-68)

More than 30 years ago, Folkman first postulated that formation of new blood vessels (angiogenesis) contributed to tumor growth in cancer. This landmark report established the field of antiangiogenic research, and investigations are ongoing into the use of antiangiogenic drugs in the treatment of cancer, in addition to other disease states characterized by excessive angiogenesis, such as rheumatoid arthritis, ocular neovascularization, and atherosclerosis. Early investigations centered on a causal role of vascular endothelial growth factor (VEGF) in tumor formation, leading to the 2004 approval of the anti-VEGF agent bevacizumab by the US Food and Drug Administration (FDA) for the treatment of colorectal cancer.

In ophthalmology, a causal role for vascular endothelial growth factor has been established in diseases of the human eye in which neovascularization and blood vessel leakage are prominent. These reports have led to clinical trials of anti-VEGF treatments to determine whether they effectively reduce the burden of choroidal neovascularization (CNV) that occurs in the most damaging form of macular degeneration—neovascular (ie, “wet”) age-related macular degeneration (AMD).

THE ROLE OF VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor comprises a family of structurally related proteins that act as mediators for angiogenesis in many experimental and clinical situations. Within this family, VEGF-A is the protein-encoding gene most responsible for ocular neovascularization. The VEGF protein mediates its action through 2 different receptors, VEGFR-1 and VEGFR-2. These 2 receptors are thought to differ in their precise roles for regulating angiogenesis, vascular maintenance, and permeability; however, both receptors are thought to be important.

Vascular endothelial growth factor is a disulfide-linked homodimer and there are many spliced isoforms. Of these isoforms, VEGF165 is the most abundant isoform and has matrix-bound and diffusible characteristics. The proteolytic breakdown products of VEGF165 include a biologically active...
product 110, which can also bind the VEGF receptors and induce endothelial cell proliferation and, presumably, vascular permeability.4

Research has indicated that VEGF is a potent inducer of angiogenesis and perhaps an even more potent inducer of vascular permeability.5 More specifically, VEGF is thought to stimulate endothelial cells to proliferate, causing them to become activated and undergo mitosis to form luminal structures. It is also a survival factor, allowing newly formed vessels to maintain themselves without undergoing endothelial cell apoptosis and vascular regression.3

Recent data have begun to emerge suggesting that VEGF may also have proinflammatory properties.6 VEGF binds to leukocytes and mediates their recruitment and adhesion to the newly formed vessels through induction of intercellular adhesion molecule 1. Leukocytes are present at the leading edge of pathologic, but not physiologic, angiogenesis. VEGF not only causes recruitment of leukocytes, but actually triggers them to produce additional VEGF, creating a cycle that ultimately amplifies the VEGF signal. In addition, leukocytes mediate endothelial cell injury by breaking down the blood-retinal barrier, increasing permeability, and promoting neovascularization.6

The ability of VEGF to induce vessel leakage derives from multiple mechanisms, including the recruitment of leukocytes, formation of fenestrae, the dissolution of tight junctions, and an increase in transcellular bulk flow. These mechanisms combine to make VEGF approximately 50,000 times more potent than histamine in inducing leakage of blood vessels.7-11 Available scientific evidence suggests that the presence in the eye of elevated levels of VEGF plays an important role in causing the abnormal blood vessel growth and blood vessel leakage characteristic of neovascular AMD. Animal models of CNV have shown it to be dependent on the presence of VEGF. An experiment by Krzystolik et al demonstrated that the anti-VEGF drug ranibizumab (formerly known as rhuFab V2) could actually block laser-induced CNV in monkey eyes.12 Although this model is imperfect because of the spontaneously remitting or short-lived nature of the lesions, the experiment was useful in that it demonstrated that the introduction of ranibizumab antagonized the angiogenic effects of VEGF and blocked the formation of new vessels.13-15 VEGF has been found to be elevated in the CNV removed from patients,16 and autopsy examinations have shown that VEGF accumulates around CNV and also in the endothelial cells.17 Whereas VEGF appears to promote neovascularization in the diseased eye, its presence in the retina of normal eyes suggests that VEGF is also necessary for physiologic ocular processes. The challenge in treating ocular disease is to inhibit VEGF selectively to destroy its pathologic function and, at the same time, to preserve its physiologic function to safeguard normal vision.

The Treatment Paradox

Retinal specialists routinely treat pathologic VEGF in patients with its neovascularization secondary to a central retinal vein occlusion. It is common practice for retinal specialists to apply laser to the ischemic retina to reduce the production of pathologic VEGF in patients with neovascular glaucoma secondary to a central retinal vein occlusion or in proliferative diabetic retinopathy, in addition to retinopathy of prematurity. However, in CNV in AMD, destructive therapies such as laser photocoagulation, photodynamic therapy, and transpupillary thermotherapy attempt to destroy the neovascularization directly rather than the VEGF-producing tissue, which is because the VEGF-producing cells are in very close proximity to the neovascularization. Therefore, whereas retinal specialists are focusing their attention on the destruction of neovascularization, the success of the therapy will also depend on whether the VEGF-producing cells are destroyed. If laser and photodynamic therapy are to be successful in eliminating the CNV, the pathologic VEGF has to be eliminated.18 Therefore, it’s not surprising that permanent destruction of the CNV results in damage to the normal tissue producing VEGF. We must assume this normal tissue is damaged; otherwise, continued VEGF production would cause additional neovascularization. Elimination of CNV and pathologic VEGF results in some loss of vision because both targets involve the central macula. The hope when using destructive therapies is that the loss of vision associated with the treatment would be less than the loss of vision associated with the continued growth and leakage of CNV (Figure 1).

If VEGF cannot be selectively destroyed to improve vision, an alternate strategy is to target the damaging effects of VEGF (Figure 2). Pharmacologic
treatment of AMD has focused on selectively inhibiting pathologic VEGF to eliminate VEGF-induced leakage and neovascularization to preserve and improve visual function. Currently, angiogenesis can be targeted at several stages, including inhibition of factors, such as VEGF, interruption of downstream signaling after receptor activation, blockade of matrix degrading enzymes, and use of endogenous inhibitors. Many of these approaches have been used in cancer treatment. In neovascular AMD, anti-VEGF strategies include the inhibition of VEGF production entirely, the binding and inhibition of extracellular VEGF, or the inhibition of the endothelial cell response to VEGF once VEGF is bound to its receptor. Various treatments are being investigated to achieve these goals.

TREATMENTS TO BLOCK EXTRACELLULAR VASCULAR ENDOTHELIAL GROWTH FACTOR

Pegaptanib sodium was approved by the FDA in December 2004 for treatment of the 3 angiographic subtypes of neovascular AMD: predominantly classic, minimally classic, and occult. Pegaptanib is a 28-nucleotide ribonucleic acid aptamer that binds to VEGF isoforms measuring 165 amino acids and larger. By binding these isoforms, pegaptanib blocks their activities. The drug’s efficacy was demonstrated in 2 concurrent, randomized controlled trials enrolling a total of 1186 patients with subfoveal CNV secondary to AMD. (For further discussion of clinical trial data, please see the article by Dr Gragoudas in this monograph.)

Several additional molecules are under investigation to block extracellular VEGF. Derived from bevacizumab, the molecule ranibizumab is nonisoform specific in its VEGF inhibition. Phase I and II clinical studies using doses from 300 µg up to 2 mg resulted in the reduction of intraretinal and subretinal fluid, inhibition of CNV growth and leakage, and improvement in visual acuity. Large double-masked randomized phase III trials, the MARINA and ANCHOR trials, are underway; 1-year results from the MARINA trial were just reported through a press release, but the data are not yet published. Approximately 95% of patients in the MARINA trial testing treatment for occult and minimally classic CNV were stable or improved. One-year results from an additional phase II study, involving ranibizumab in combination with photodynamic therapy versus ranibizumab alone, were also just announced with approximately 90% of the combination patients stable compared to only approxi-

Figure 1. The Great Treatment Paradox?

By destroying the CNV, we also destroy the tissue producing VEGF

By destroying the tissue producing the VEGF, we also damage the vision


Figure 2. Effects of Vascular Endothelial Growth Factor

Optical coherence tomography-3 image through fovea of a patient with choroidal neovascularization secondary to age-related macular degeneration. Subretinal fluid accumulation in the macular edema. ME = macular edema; PED = pigment epithelial detachment; SRF = subretinal fluid. Used with permission from Philip J. Rosenfeld, MD.
approximately 60% of the photodynamic therapy-only eyes.\textsuperscript{22}

Other topics under investigation are whether injections should be based on a strategy using a fixed-dosing interval or a strategy based on dosing only when there is evidence of CNV leakage.

Another drug being investigated is bevacizumab, an anti-VEGF agent indicated for use in metastatic colorectal cancer. Bevacizumab has the same anti-VEGF binding properties as ranibizumab because ranibizumab was derived from bevacizumab, and both drugs bind all VEGF isoforms with high affinity. However, the two drugs differ by how they are administered. Whereas ranibizumab is injected into the eye, bevacizumab is administered intravenously every 2 weeks. Before the approval of pegaptanib in 2004, no treatments were proven effective for all 3 subtypes of neovascular AMD, prompting consideration of bevacizumab as an experimental therapy. The Systemic Avastin for Neovascular AMD study was a nonindustry sponsored clinical investigation. Preliminary results from a small number of patients indicate that bevacizumab performs the same or better than early reports for ranibizumab.\textsuperscript{23} Although an advantage of the drug is that it does not require ocular injection, systemic administration may result in higher risks of side effects. Whether benefits outweigh the risks will require a larger study, but hypertension was the only risk identified thus far.

A VEGF blocker termed the “VEGF-Trap” is a fusion protein consisting of domains 2 and 3 from VEGF receptors 1 and 2.\textsuperscript{24} By combining these proteins, the VEGF-Trap blocks access of all VEGF isoforms to the cell membrane, with an estimated affinity 1000-fold higher than the molecule bevacizumab.\textsuperscript{25} Clinical trials assessing intravenous administration of the agent in neovascular AMD are ongoing.

**TREATMENTS DESIGNED TO BLOCK ENDOTHELIAL RESPONSE TO VASCULAR ENDOTHELIAL GROWTH FACTOR**

A myriad of molecules that block the intracellular action of VEGF mediated through its receptor are under investigation. These agents are designed to block specific pathways by which VEGF exerts its effect on endothelial cell permeability, survival, migration, and proliferation.

A phase I open-label, dose-escalation trial is testing Sirna-027, a chemically modified short-interfering RNA molecule that targets VEGFR-1 and is administered by...
intravitreal injection. To view an animation of how small-interfering RNA molecules function inside the cell, go to www.sirna.com/sirnascience/rnai.html. Several pharmaceutical manufacturers are also investigating a potential role for protein kinase-C inhibitors and other tyrosine kinase inhibitors, administered locally or systemically, for the treatment of neovascular AMD. Although this is an active and abundant area of clinical investigation, data have not yet been reported.

TREATMENTS TO BLOCK VASCULAR ENDOTHELIAL GROWTH FACTOR PRODUCTION

The use of small-interfering RNA targeted to block VEGF expression is in phase I clinical trials. These investigations are evaluating the use of a single RNA interference drug molecule (Cand5), administered by intravitreal injection, to determine its efficacy in blocking the production of VEGF protein molecules. Other small-interfering RNA anti-VEGF molecules are being evaluated in preclinical studies.

CONCLUSIONS

Data from animal and human studies show that neovascularization requires the presence of VEGF. These data have prompted a wide range of scientific inquiry into a variety of therapeutic treatments aimed at blocking production of VEGF, blocking its extracellular effects, or the endothelial cell response. All but one of these treatments, pegaptanib, remain investigational at this time.

Overriding objectives of anti-VEGF therapies are inhibiting angiogenesis, permeability, and inflammation. The medical community eagerly awaits outcomes from these ongoing investigations. It is possible that in the future, treatments will involve a combination of agents, including not only antiangiogenic antipermeability agents but possibly corticosteroids and photodynamic therapy. For those of us who see patients who are experiencing continued vision loss from neovascular AMD, new and emerging options are generating much excitement and renewed hope.

DISCUSSION

Dr Schachat: This morning we heard a presentation on the first 9 patients to receive a systemic anti-VEGF drug for treatment of neovascular AMD. Bevacizumab is FDA approved for use in patients with colon cancer (fewer than 3 lines of visual acuity. This percentage increased to 57% when treatment interval and drug reflux were taken into account. However, the number of patients in this subgroup analysis is small and the difference was not statistically significant. AA and the administration technique of posterior juxtascleral depot were generally well tolerated. In May 2005, the US Food and Drug Administration issued an approvable letter for AA's New Drug Application. The results of additional studies, which are underway, will be needed for final approval.

Squalamine lactate. This synthetic small molecule, delivered intravenously, is selective for activated endothelial cells. Its long intracellular half-life and rapid systemic clearance allow for intermittent dosing. A phase I open-label study of 40 patients with any CNV lesion subtype was conducted primarily to evaluate safety, with secondary endpoints of visual acuity and effects on angiography. Squalamine was administered by multiple intravenous infusions over a 1-month timeframe. Visual acuity assessments showed that at 2 weeks, 25% of eyes benefited with a 3-line or more improvement, increasing to 33% by 4 weeks. At the end of 2 months, vision continued to be stable in 97% of eyes. At 4 months, vision improvement dropped to a rate of positive change in 26% of eyes, although 100% of patients showed stabilized vision. A subsequent pharmacokinetic study of a 40-mg drug dose reported a positive 3-line change in visual acuity in 1 of 6 patients (17%) from baseline to 4 months. At study end, vision was stabilized in all of the study subjects. Investigators are currently enrolling for larger phase II studies, and phase III trials may begin by the end of 2005.

REFERENCES

every 2 weeks. Although there are no ocular side effects associated with this drug, there are some cardiovascular risks. However, intravitreal agents, administered locally as opposed to systemically, are associated with endophthalmitis, retinal detachment, and cataract.

In general, it will be difficult for patients to calculate the risks of one treatment over another. Perhaps a decision analysis tool could help patients with treatment choices by weighing degree of concern against degree of risk. The choice will depend on the individual patient who must decide whether they are more concerned with ocular risks or risks to overall health with systemic therapy.

Dr D'Amico: At the present time, systemic therapy with any available anti-VEGF agent is not an attractive option. In the verteporfin trials, no systemic side effects were reported. There is no reason to dose a patient’s heart, kidney, liver, and lungs with experimental agents to achieve ocular benefits when those benefits can also be achieved with therapies administered locally with intravitreal or periocular therapy. We have even determined that vitamin therapy presents a risk of lung cancer and genitourinary side effects in some patients. Therefore, I think systemic therapy needs to be tested rigorously.

Dr Rosenfeld: I agree. When the bevacizumab trial was initiated, patients had no other options. Currently, in the bevacizumab trial, all patients enrolled were given the option to switch to pegaptanib. This is the only ethical position to take. Given the risks of systemic therapies, bevacizumab should not be first-line therapy, but it is still beneficial to have a variety of treatment options available to us. Some patients may respond well to all of the therapies, but others may respond to only one, and possibly none. We don’t have enough evidence to indicate what the outcome will be with any given treatment.

I think in offering patients a progression of treatment options, I would always initiate therapy with the safest option. If the patient shows no improvement, I would offer another option. Based on my experience with anti-VEGF therapies, in particular ranibizumab, I expect the fluid to be resorbed and the vision to improve. If that doesn’t happen, then I’ll look for another option for my patients. Patients can make informed decisions when they are advised of the potential risks and benefits associated with each therapy, even when final phase III results aren’t available for a particular treatment. Pegaptanib, an intravitreal injection, is the safest treatment option, and my primary treatment of choice right now. However, pegaptanib may not be the most beneficial. Fortunately, other treatment options are available to patients who do not respond well to pegaptanib, and they may also want to participate in clinical trials.

Dr Gragoudas: I would not advocate having patients make treatment decisions. The physician should recommend the appropriate treatment for the individual patient, depending on many contributing factors. The systemic therapies currently under investigation need to be proven as effective and safe as approved treatments. However, ultimately, the physician must be the one who advises which treatment is best.

Dr Ha: Safety should be the primary consideration; efficacy is secondary. The safety of bevacizumab in the ophthalmology setting is unknown. It has been associated with a thromboembolic event rate of 4.4% in patients with cancer treated at high doses at 2-week intervals, and we have not yet determined the lowest tolerable and therapeutic dose. I agree that physicians should make recommendations, as the patients simply cannot understand all of the factors that need to be weighed in the decision. But again, patients value vision, sometimes even more than life. We have a tremendous opportunity for development of more efficacious treatments in the future and we should be open-minded to exploring the options—keeping safety paramount.

REFERENCES


